for TRO-BMD, *IGSF11*). We also replicated two loci 3p21 (*rs148725943*, discovery p=6.61x10-7, replication p=5.22x10-4 for TRO-BMD, *CTNNB1*) and 8q24 (*rs7839059*, discovery p=2.28x10-7, replication p=1.55x10-3 for TRO-BMD, *TNFRSF11B*) that were reported previously.
**Conclusion**: Our findings provide useful insights that enhance our understanding of bone development, osteoporosis, and fracture pathogenesis.
http://dx.doi.org/10.1016/j.jot.2016.06.083

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### PHOTODYNAMIC THERAPY (PDT) TO ENHANCE HEALING OF FEMUR FRACTURES WITH A CRITICALLY SIZED DEFECT

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**Introduction**: The majority of long bone fractures heal successfully without complications; however fractures resulting from high impact trauma can result in delayed healing or non-union. Early intervention could decrease patient morbidity and reduce health care system costs. Photodynamic therapy (PDT) is a minimally invasive local treatment involving administration of a photosensitizer, which is activated by laser light leading to the production of singlet oxygen, which can induce apoptosis and/or necrosis of targeted cells and tissue and also influence immune responses. PDT treatment of metastatically involved vertebrae resulted in improved vertebral bone strength, stiffness and architecture, motivating studying PDT as an approach to augment bone healing. The aim of this study was to evaluate the ability of PDT treatment to enhance healing in fractures exhibiting critically size defects.
**Materials and Methods**: Femoral fractures with critically sized defects (6 mm) were generated in 30 adult female Sprague-Dawley rats (7 or 15 week survival). Under general anaesthesia an 8-hole PEEK plate was attached laterally to the femur. Using a Gigly saw, a bone piece was removed followed by closure of musculature and skin. Rats were randomly allocated to three groups: control, PDT applied either 1 day, or 7 days post fracture. A photosensitizer (Visudyne, Novartis, Canada) was injected (1mg/kg) followed 15 minutes later by light delivery (75J; 690 nm) using a 1 cm diffuser fibre placed parallel to the fracture. The rats were euthanized 7 or 15 weeks after induction of the fracture. μCT images of the femur at an isotropic 13.3 μm/voxel resolution (Inveon MicroCT, Siemens, Germany) were acquired and analysed (AmiraDev 5.2, FEI Visualization Science Group, USA). Thereafter, the bone was decalcified and processed for histology. Statistical analysis was performed using a 1-way ANOVA.
**Results**: All rats recovered well; however five animals were euthanized early due to plate displacement. The total bone volume (TV) evaluated from μCT images within the fracture gap did not show significant differences. In contrast, BMD (gHA/cm$^2$) trended toward higher values in the PDT treated groups compared to controls. The fracture gap measured on μCT images of the 7 week group demonstrated a trend toward smaller gaps in the PDT treated groups ($p = 0.0535$). A statistically significant ($p = 0.0085$) smaller gap is present in the PDT treated groups after 15 weeks. Histology of the control group showed more cartilage and woven bone formation in contrast to the PDT treated groups which exhibited more structured and mature bone.
**Discussion**: PDT treatment of rat femur fractures led to lower overall formation of bone, but the bone had higher density with a decrease in the size of the fracture gap. The increase in bone density in the PDT treated groups may suggest formation of better quality bone (vs. quantity of bone). Histologically, with more cartilage and woven bone present in the control group in contrast to more mature bone and in the PDT group, the fracture healing seems to follow a different pattern, which requires further investigation.
**Funding/support**: Congressionally directed Medical Research programs (CDMRP), grant # OR090260.
http://dx.doi.org/10.1016/j.jot.2016.06.084

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### PATHOPHYSIOLOGY OF CHEMOTHERAPY-INDUCED DAMAGE OF BONE MARROW MICRO-VASCULATURE AND POTENTIAL PROTECTIVE EFFECTS OF FLAVONOIDS IN RATS

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**Introduction**: It has been widely shown that bone formation and remodelling would not occur unless there is already a properly established micro-vasculature. However, blood vessels can be damaged by extrinsic causes such as chemotherapeutic agents. Methotrexate (MTX) is an anti-metabolite chemo-agent widely used in treatment of many diseases including childhood leukaemia and inflammatory disorders. While previous studies showed that MTX can cause long-term skeletal side effects, whether and how it damages bone marrow micro-vasculature remains unclear. Using a rat model and endothelial cell-culture models, we addressed these questions. In addition, since we recently showed that the osteogenic, anti-oxidant, and anti-inflammatory flavonoid genistein can protect bone in MTX-treated rats, here we also investigated effects of genistein in the recovery of damaged blood vessels in rats treated with MTX. Furthermore, we also examined potential treatment effects of genistein and a related flavonoid, icariin, on viability and tube-formation ability of endothelial cells treated with MTX *in vitro*.
**Methods**: Animal studies: To study the effect of MTX on blood vessels, groups of male (6-week-old) Sprague—Dawley rats were subcutaneously injected with MTX (0.75mg/kg) once daily for 5 days and were sacrificed on day 1, 3, 6, 9, 11, and 14. To study the protective effects of genistein, in some MTX-treated rats, genistein was administrated by oral gavage (2 mg/100 g BW) for the whole period starting from day 0 until one day before kill (day 9). Treatment effects on number and sizes of bone marrow micro blood vessels were examined histologically in tibiae. MTT viability assay: Concentration-/time- dependent treatment effects of MTX (10nM-10μM) were examined on viability of cultured rat sinusoid endothelial cells (SECs) and effects of 24 hour treatment with MTX plus icariin/genistein (10nM-10μM) were also studied. Apoptosis detection by flow cytometry: SECs were treated with MTX (1μM/mL) for 24 and 48 hours and apoptosis was detected based on their cell surface Annexin-V expression. Tube formation assay: SECs were treated with/without MTX (1μM), icariin or genistein (100nM-10μM) and effects on angiogenesis were examined based on formation of tubes by SECs on Matrigel.
**Results**: Histological image analyses of H&E-stained tibial sections showed significant blood vessel damage in the bone marrow of rats on days 6 and 9 and significant but partial recovery on days 11 and 14 following the first MTX dose. Histology analyses suggested that genistein potentially attenuates MTX-induced blood vessel damage in the bone marrow. Examining any cytotoxic effect of MTX on endothelial cells, MTT assays showed that the viability of SECs was not affected after 24 hours of treatment with MTX (10nM-10μM). However, following 48 hour treatment, viability of SECs was reduced in a concentration-dependant manner. Flow cytometry analysis revealed that SECs underwent apoptosis following 48 hours (but not 24 hours) treatment with MTX (1μM). MTT assays also showed that neither genistein nor icariin treatment affected viability of SECs viability. Tube formation assays showed a reduced tube formation potential of SECs treated with MTX (1μM). Interestingly, icariin or genistein (10μM)-treated SECs showed enhanced tube formation and icariin or genistein treatment can prevent MTX-induced decrease in tube formation.
**Discussion and Conclusion**: Our *in vivo* and *in vitro* studies suggest that MTX causes blood vessel damage in the bone marrow, potentially by inducing apoptosis in endothelial cells and also interfering in the process of angiogenesis. Our *in vitro* tube formation assays showed that icariin and genistein might not only promote angiogenesis but possess some protective effect against MTX damage. Consistently, our *in vivo* studies also showed some positive effects of genistein treatment in reducing MTX-induced blood vessel damage in the bone marrow of rats.
http://dx.doi.org/10.1016/j.jot.2016.06.085

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### INCREASED EZH2 COMBINED WITH DECREASED OSTEOBLASTOGENESIS IN LOCAL IRRADIATION INDUCED RAT BONE LOSS

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Radiation therapy is a common treatment for cancer patients. The adverse effects are the insufficiency fractures and bone loss. Epigenetic regulation plays an important role in the BMSCs differentiation. We reported here, the changes of local bone after a single-dose $^{137}C_S$ irradiation exposure in rats. The bone mineral density (BMD) of the femur and the trabecular bone volume in the tibia were significantly decreased at 12 weeks after irradiation. The micro-CT results showed that the tBMD, Tb.h, and Tb.N were also significantly reduced after 12 weeks of irradiation exposure. The ALP-positive OB.S/BS was decreased by 42.3% after 2 weeks irradiation, and decreased by 50.8% at the 12 weeks. In contrast to the decreased expression of Runx2 and BMP2, we found EZH2 expression was significantly increased after 2 weeks of single-dose $^{137}C_S$ irradiation in BMSCs. In conclusion, our results demonstrated that the single-dose $^{137}C_S$ irradiation induces the loss of BMD and bone micro-architecture deterioration in rat skeleton, as well as the increased expression of EZH2 and decrease of osteoblastogenesis after irradiation. The underlying mechanisms may be required to further investigate the relationship.
http://dx.doi.org/10.1016/j.jot.2016.06.086

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### MYRICITRIN INHIBITS OSTEOCLASTOGENESIS

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Myricitrin is a botanical flavonol glycoside, extracted from leaves of *Myrica cerifera* and other plants. Abundant evidence supports myricitrin has anti-oxidative, anti-inflammatory, and neuroprotective effects. Osteoclastic bone resorption is